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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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Christopher	Chen	Baltimore, MD

Additional inventors are being named on the ____ separately numbered sheets attached hereto

Producing 1

TITLE OF THE INVENTION (500 characters max)

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ENCLOSED APPLICATION PARTS *(check all that apply)*

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☐ CD(s), Number ____
☐ Drawing(s) *Number of Sheets* ____
☐ Other (specify) ____
☐ Application Data Sheet. See 37 CFR 1.76

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Respectfully submitted,

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REGISTRATION NO. 45,282 *(if appropriate)*
Docket Number: 4151

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Docket Number 4151

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U.S. Provisional Patent Application

JHU Ref. No. DM-4151

Producing Arbitrary Arrays of Particles using Dielectrophoresis

Inventors: Chen, Gray, Tan, Voldman

INVENTION DESCRIPTION

Describe the invention completely, using the outline given below.

1. Abstract of the Invention [Briefly describe the invention]

Recently, the need for precisely positioning cells in micrometer-scale cultures has arisen in the miniaturization of experimental systems. Such miniaturization leads to reductions in cost of reagents and in consumption of valuable cells, allows for automated handling of experiments, and increases speed of experiments. Miniaturized cell culture systems are being used to increase the speed of discovery in basic cell biology, to construct cell-based devices, and to explore the production of engineered tissues. The current invention uses dielectrophoresis (DEP) to simultaneously position large numbers of cells in arbitrary planar coordinates with single cell precision. Such patterning of cells is independent of the chemistry and topology of the surface on or near which the array is formed. The same DEP-based technique used to pattern cells can also be applied to positioning one or more types of other solid, semisolid, or liquid particles in arbitrary arrays with single particle precision.

2. Problem Solved [Describe the problem solved by this invention]

Several passive methods are available to create arrays of cells or other particles on a surface. These methods generally fall into two categories: chemical patterning in which adhesive and non-adhesive areas are patterned onto a physical surface, and topological patterning in which wells or other topological regions are formed on a surface. A suspension of cells is then flowed onto these substrates, such that cells settled randomly onto the surface and either are 'trapped' by the surface or are not, depending on the location each cell lands. Thus, both methods control the regions in which cells or other particles remain using passive approaches, and result in random statistical distribution of the cells or particles across the surface. Thus, the methods cannot precisely position individual cells or other particles, and further cannot pattern multiple types of cells or other particles in precise stoichiometry at specific regions of the substrate. In contrast, this invention uses forces generated by dielectrophoresis to actively move individual cells or other particles to precise locations. Because this is an electrical force that can be reproduced many times across a surface, it can be used to move many cells (thousands to millions) simultaneously. Therefore, arbitrary patterns and stoichiometries of multiple cells types, or multiple types of other particles, can be formed using this active method. Because dielectrophoresis-based trapping (DEP trapping) is orthogonal to surface chemistry and topology, it can also be used to confine cells or other particles to subsets of adhesive regions or depressed wells.

There are numerous applications for using DEP to position large numbers of cells or particles into arrays. No tool currently exists which can position large (>1000's) numbers of small particles into an arbitrary array with the ability to position the particles individually or in groups. The integrated circuit industry needs such a tool to position large numbers of small, prefabricated components on a circuit. The medical diagnostics industry needs this tool to quickly and efficiently construct cell-based devices to precise specifications. The military needs this tool to construct cell based biosensors for the detection of biological and chemical warfare agents. In general, any industry which requires the assembly of particles too small to effectively array with robotics or by human hands, or any industry which needs to position large numbers of particles simultaneously in an arbitrary array, will find DEP trapping useful.

3. Novelty [Identify those elements of the invention that are new when compared to the current state of the art]

There is no current system to precisely and simultaneously position an arbitrary array of single or multiple types of small (<1mm) particles on or near a planar or non-planar surface independently of the surface's chemistry and topology. Other methods, such as inkjet delivery of reagents, or laser tweezers, are serial methods. That is, they can only deliver compounds to one location at a time. Although DEP was thoroughly described in 978 [1], it has never been used for arraying of cells in an arbitrary pattern. Thus, we believe that this invention is both novel and non-obvious.

Patent Search: Searched delphion.com patent database 10.9.02 for all dates

Searched: dielectrophoresis, 64 results
Searched: dielectrophoresis and array, 14 results, dealing with particle separation using DEP or causing particles to react (collide) using DEP.
Searched: dielectrophoresis and pattern, 2 results, one for a fluid pumping scheme and another for a printing device.

Most of the patents dealing with dielectrophoresis involve pumping, separations, particle collisions, or other uses not related to trapping/patterning. The closest patent we've found (US #5355577 Michael B Cohn, Method and Apparatus for the assembly of microfabricated devices) uses the charge leakage through holes in one plate of a parallel-plate capacitor to generate dielectrophoresis and trap particles. The geometry of this invention is inherently different from, and less useful than, the "posts-and-lid" geometry developed in our work. In patent 5355577, particles are trapped outside of the two plates. In "posts-and-lid," particles are trapped between the two electrodes. This geometrical difference allows "posts-and-lid" to operate at much lower voltages (5V versus 8 kV in the respective preferred embodiments), and therefore be much safer and more economical. Patent 5355577 only proposes the trapping of particles for integrated circuit manufacture, and does not mention any biological applications. In patent 5355577, no method of independently actuating the traps is feasible. Patent 5355577 does not detail the combination of DEP trapping with other patterning, such as patterned adhesiveness. Finally, patent 5355577 suggests only gravity as a destabilizing force to remove untrapped particles, and does not suggest fluid flow, magnetic forces, etc.

4. Detailed Description of the invention:

On a separate page(s), attach a detailed description of how to make and use the invention. The description must contain sufficient detail so that one skilled in the same discipline could reproduce the invention. Include the following as necessary:

1- data pertaining to the invention;
2- drawings or photographs illustrating the invention;
3- structural formulae if a chemical;
4- procedural steps if a process
5- a description of any prototype or working model;

In general, a manuscript that has been prepared for submission to a journal will satisfy this requirement.

SEE ATTACHED

Workable Extent/Scope:

We have demonstrated that different types of cells can be patterned into arbitrary arrays using dielectrophoresis.

Arrays of cells positioned using dielectrophoresis are highly controllable. One single cell is trapped at each electrode when electrode dimensions are smaller than cell diameters, allowing single cell precision in the pattern formed.

In the case that the trapping of multiple particles at each electrode is desired, electrodes larger than 1 particle diameter can trap multiple particles.

Various techniques to pattern surface chemistry can be combined with DEP patterning to further influence or restrict cell location and locomotion after DEP trapping. These techniques include, but are not limited to, microcontact printing, protein adsorption, and lithography-based techniques.

DEP cell trapping does not harm cells, as demonstrated by their normal proliferation and morphology.

The electrodes used for DEP trapping could also be used to stimulate cells electrically or record electrical signals from the cells. For example, a culture of cardiac cells could be stimulated at select locations. A functional network of neuronal cells, being unpatterned or patterned with DEP, could be stimulated with electrical impulses delivered via the DEP electrodes and then the propogation of these signals could be analyzed via DEP electrodes.

Because DEP is activated by electric impulses, the traps can be arbitrarily turned on or off individually or in groups. Thus, we can not only trap cells at will, but also release them at any given time. This could be useful in systems that need to capture a cell temporarily to analyze it, and release it depending on analysis results (such as a FACS sorter or system to collect rare cells from a mixed population).

The electrodes used for DEP trapping could be used to lyse or electroporate adherent cells, either selectively or throughout the substrate. Electroporation of cells on the electrodes could facilitate transfection, either selectively or throughout the substrate.

DEP trapping can be used to align cells or other particles with detection or stimulation elements. Such detectors or stimulators may be optical, chemical, electrical, mechanical, magnetic, or thermal, or be based on other principles.

DEP is actuated by electricity, and is therefore easily integrated with computer control and other electronics.

DEP trapping can be actuated by low voltages (<5V) and can therefore be controlled by standard CMOS circuitry.

DEP trapping is applicable to solids, semisolids, or liquids suspended in liquids or gasses, provided that the suspended particle or droplet has different electrical characteristics than the surrounding substance.

DEP trapping of arrayed cells can occur in parallel, with the entire array being trapped simultaneously.

The electrodes can be wired to be controlled individually, so that each DEP trap on a substrate can be actuated independently of the others, to array cells serially, with individual cells or groups of cells being

trapped at different times.

DEP can place cells or other particles on top of a cell layer or other intervening layer, allowing 3D constructs to be built from multiple layers.

As demonstrated by functional DEP trapping with or without a silicon dioxide passivation layer, trapping can occur with direct contact between cells and electrodes or with an intervening layer, which may serve to provide uniform surface chemistry and/or protect the cells from potential adverse effects of direct contact with active electrodes.

Precise, single cell positioning by DEP allows cell alignment with detection and/or stimulation elements during the construction of cell-incorporating devices, such as biosensors.

Multiple types or groups of cells or other particles can be patterned on the same substrate using DEP, through independent actuation of individual traps of groups of traps. For example, cells of type A could be trapped on electrode set A, while other electrodes present were not actuated. After washing away untrapped cells of type A, cells of type B could be trapped on newly actuated electrode set B. Cells of type A could be held on electrode set A by cell adhesion forces and/or continued actuation of electrode set A. This process could be repeated with cells of type C and so on. Trapping of multiple cell types will facilitate the construction of automated cell screens and the study of both homotypic and heterotypic cell interactions.

DEP can facilitate the patterned delivery of non-cellular particles, such as beads, DNA, RNA, or proteins, to cells or other biological or non-biological elements present on the substrate.

DEP trapping of multiple types of cells can facilitate combinatorial biology in which the interactions between every possible combination of several cell types are analyzed simultaneously, allowing for high throughput experimentation.

DEP trapping can be used to deliver non-cell particles to cells patterned with DEP of by other means. For example, multiple types of beads loaded with bioactive, or potentially bioactive, compounds could be positioned many cells or groups of cells. The response of these cells of groups of cells to these compounds could then be assayed in parallel, allowing for high throughput data collection. One or more compounds could be delivered to each cell or group of cells.

Fluid (gas or liquid) flow may be used as a destabilizing force to remove untrapped cells from a substrate following DEP patterning.

Gravity may be used as a destabilizing force to remove untrapped cells from a substrate following DEP patterning. The effective force due to gravity may be increased with centrifugation or other acceleration.

Magnetic attraction or repulsion may be used as a destabilizing force to remove untrapped cells from a substrate following DEP patterning.

Vibration or other acceleration may be used as a destabilizing force to remove untrapped cells from a substrate following DEP patterning.

Both positive and negative DEP may be used to position arrays of cells.

DEP traps may be fabricated as part of planar or non-planar surfaces.

A variety of construction methods may be utilized to achieve the "post-and-lid" electrode geometry used to trap cells or other particles with DEP.

The "post-and-lid" electrode geometry used to trap cells or other particles with DEP will function using a variety of dimensions, including variations in the dimensions of chamber height, electrode height, electrode width, and electrode spacing.

Because DEP trapping is orthogonal to surface adhesiveness and other surface chemistry, DEP can trap cells only on a subset of adhesive areas. This phenomenon will be useful for sensor construction, as cell-free adhesive areas can be used to provide a reference signal with which to compare cell-containing areas.

DEP trapping can be used to create defined starting positions for cells whose locomotion is not confined after trapping.

DEP trapped particles need not be adhered to the surface containing the traps. For example, particles can be trapped before the electrodes are inverted and then turned off. Particles which are heavier than the surrounding media will fall onto an opposing surface. This phenomenon greatly expands the selection of surfaces on which to place or adhere the trapped/patterned particles. In the case that particles are lighter than the surrounding media, the DEP substrate may trap the particles while inverted (facing downward) and then be flipped over, before turning the traps off. Once the traps are turned off, the light particles will rise and contact the opposing surface.

DEP trapping of cells can place a majority of cells in any desired arrangement. Although other techniques such as patterned surface chemistry will place a minority of cells in a desired arrangement, this limits analysis of such correctly placed cells to *in situ* assays. With DEP, since a majority of cells are placed correctly, the cells can be pooled for bulk assays such as Western Blots, Southern Blots, Northern Blots, and ELISAs.

Because DEP trapping allows precise control over cells number, the technique will greatly increase the integrity of the data collected from sensors or other devices whose outputs are proportional to cell number, by reliably controlling this number.

DEP can be used to create an arbitrary and controllable stoichiometry of particles in a given region. Physical walls, wells, or a discontinuity in the surrounding media can then be used to further separate such regions.

DEP trapping of one or more cell types can be used to create functional biological constructs, such as a functional nerve chip or other construct where biological function depends on correct cell position, geometry, or type.

DEP trapping can be used to create arrays of particles smaller than 0.1 nm in size, 0.1-10 nm in size, 10nm to 100 nm in size, 100nm to 1 micron in size, 1 micron to 10 microns in size, 10 microns to 100 microns in size, or 100 microns to 1000 microns in size, larger than 1000 microns in size, or composed of multiple sizes.

DEP trapping can be used to create arrays of 1-10 particles, 10-100 particles, 100-1000 particles, 1000-10000 particles, 10,000-100,000 particles, 100,000-1,000,000 particles, 1,000,000-100,000,000 particles, or more than 100,000,000 particles.

DEP electrodes may be fabricated from metals or other conductive materials.

DEP trapping allows the use of both DC and AC electric fields. DEP trapping allows the use of both low and

high frequency fields. With high frequency fields, electrical effects on cell membranes are minimized, allowing the application of relatively strong electrical fields without cell health effects.

After DEP trapping, cells or subcellular areas can be exposed to different stimuli than other cells or subcellular areas.

All aspects of DEP trapping detailed in relation to cells can be applied to other solid, semisolid, of liquid particles.

The electrodes used to trap cells using DEP can also be used to lyse a predetermined or selected population or subpopulation of cells for subsequent analysis with a standard or nonstandard biological assay, while excluding non-lysed cells. Alternately, cells left behind after other cells are lysed can be removed via electrical lysis or other methods such as trypsin treatment. In the case or cell removal via trypsin or other non-destructive methods, the cells can be subsequently analyzed or cultured on the same surface or another surface, or in solution.

The counter electrode ("lid") placed near the "post" electrodes which are used for particle trapping need not be continuous. The "lid" electrode might, for example, be in the form of a mesh or separate elements, which would permit the passage of particles and the surrounding media, or only the surrounding media.

DEP-based trapping provides a method by which the initial locations of cells or other particles on are surface are determined by the locations of active traps. Later, the positions of particles of cells may remain the same or be determined by DEP or other forces.

The "post-and-lid" apparatus described for performing DEP-based trapping can be fabricated by any method which produces a suitably similar device.

As shown by our proof-of-concept demonstrations, the "post-and-lid" apparatus can be manufactured easily using existing technology.

Detailed Description:

Summary:

This work demonstrates the use of dielectrophoresis [1] to position arbitrary arrays of cells with single cell precision. These efforts address the growing need for precise and robust methods to position biological particles, such as cells. Such positioning is useful for studying the ways in which cells adhere to surfaces, interact with other cells of the same and different types, and migrate in response to a variety of stimuli.

The commercial sector will benefit from the ability to pattern multiple cell types in order to creating functional tissue engineering constructs, such as fully functional nerve chips. Additional commercial interest will stem from the ability to position cells on or within biosensors and other cell-based devices, allowing construction to exacting design specifications, including the alignment of cells with detection and stimulation elements comprising the device, especially in the case of MEMS (MicroElectroMechanical Systems). With an increased interest in the analysis of individual cells, arrays of individual cells will allow such analysis to proceed in a massively parallel, high throughput format.

Background and Significance:

Recent advances in microfabrication technology have facilitated increasingly sophisticated control over the cellular microenvironment [2]. Precisely tailoring the chemical and physical nature of the surroundings of cells has led to a greater knowledge of how these cues influence proliferation, differentiation, and death [3]. However, current techniques lack the ability to easily pattern multiple cell types or actively position the cells.

By controlling the distribution of cells with electrical forces, we were able to engineer cell cultures in a more complicated and precise manner than was previously possible. For example, cells could be initially positioned in a straight line to facilitate straightforward analysis of cell migration on a population level. We can then vary the density of cells on the line to examine how cell-cell contact affects the migration response. With the addition of adhesiveness (surface-chemistry) patterning, cells could be placed by DEP in a subregion of the adhesive areas to observe spatially directed spreading or migratory behaviors. With arrays of dielectrophoresis-based traps, the biological effects of cell packing can be studied. Because the electrodes driving dielectrophoresis (DEP) can be independently energized, multiple cell types could be patterned separately (Figure 9).

In order for DEP to function as an effective patterning technique, cells must be manipulated without adverse effects. As others have observed, cells are able to withstand electric fields of suitably low amplitude and high frequency [4] We did not observe adverse health effects, even when cells were subjected to DEP at higher voltages and longer periods than were necessary for patterning.

Cells to be analyzed for potential toxic effects were subjected to DEP at 10 volts for 5 minutes. At this point, the DEP device was removed from the power source and placed in a tissue culture incubator. Cells were inspected after 15 hours and again after 72 hours. No unusual blebbing, vesicles, or other morphological evidence of adverse health effects were observed. Cell division rates were similar to tissue culture controls, indicating that cell health was not affected by DEP or other manipulations involved in the patterning process (Figure 4).

The integration of living cells into bioMEMS requires precise and robust processes to pattern cells in alignment with the analytic components of these devices. Currently, the simultaneous positioning of large

numbers of cells typically relies on three techniques: patterning surface chemistry such that cells preferentially adhere to adhesive regions, allowing cells to settle into depressed wells, or controlling fluid flow such that cells are localized only to certain streams and therefore certain regions of the substrate. None of these methods can control the placement of individual cells. We describe a method to move living cells safely and rapidly to precise locations using electrodes constructed with standard microfabrication techniques. Small potentials applied to the electrode array result in dielectrophoresis (DEP), the force on polarizable bodies in a nonuniform electric field. Positioning cells using electrical forces is inherently well suited for integration into computer controlled, chip-based devices. We will demonstrate the use of DEP to form arbitrary arrays of single cells on planar surfaces within a few minutes. In some cases, the substrates are patterned with aligned adhesive regions. Data demonstrating the minimal effects of this patterning approach on cell health will also be presented. Potential applications range from functional nerve chips to automated cell interaction screens.

DEP functions by exerting electrical forces of particles which may be either charged or electrically neutral. (See Figure 1). When such a particle enters an electrical field, it becomes polarized. If the electrical field is non-uniform, a particle which is more polarizable than the surrounding fluid or gaseous media is pulled toward the stronger region of the field, undergoing positive DEP. A particle which was less polarizable than the surrounding fluid of gaseous media would be pulled towards the weakest region of the electric field, undergoing negative DEP (not shown).

DEP has been successfully used to manipulate particles, ranging from 10 nm to 100 microns, in this manner. However, most previous attempts have only been able to control the general position of large groups of cells or other particles[5], and unable to precisely position individual cells or other particles. Additionally, these general positions are subject to the geometries which are utilized to create the non-uniform electric field(s). Only one previous approach has achieved single-particle specificity [6]. However, due to the multiple (4) electrical connections required for each trap, these traps have only been applicable to limited 1 dimensional arrays, i.e. lines, of trapped particles. Additionally, this previous approach requires electrodes which protrude significantly past the plane of the substrate. These protruding electrodes interfere with the fluid flow used as the destabilizing force, and would complicate analysis of cell behavior, were such an approach used to study cell locomotion after adhesion to the substrate. Finally, a non-planar surface precludes or interferes with the use of a variety of techniques commonly used to modify surface chemistry, such as microcontact printing and lithography. Therefore, only the current method is able to achieve single particle trapping in a format which is arrayable and combinable with other patterning techniques, such as patterned surface chemistry. The two-layer, "posts-and-lid" geometry involved in the current technique makes it uniquely suitable for the production of arbitrary arrays, since particle positions are not restricted by a the geometry used to create the non-uniform electric field needed to achieve DEP.

Because most cell types become motile after they adhere to a planar surface, we have combined DEP patterning with patterned substrate adhesiveness in order to preserve the fidelity of the pattern after cells adhere to the substrate. Cell adhesion to a surface after DEP trapping has not previously been demonstrated, with our without the addition of adhesiveness patterning.

Materials and Methods:

NIH/3T3 fibroblasts (3T3s, ATCC CRL-1658) and bovine pulmonary arterial endothelial cells (BPAECs, VEC Technologies, Rensselaer, NY) were cultured under 5% and 10% CO_2 atmospheres, respectively. All cells were cultured in Dulbecco's modified Eagle's media supplemented with 10% calf serum, 100 U/mL penicillin, and 100 μg/mL streptomycin (Life Technologies). Prior to plating on experimental substrates, cells were detached using 0.25% trypsin and 1 mM ethylenediaminetetraacetic acid (EDTA). Prior to being subject to dielectrophoresis trapping, cells were resuspended in DEP Media consisting of 300 mOsm sucrose with 1% calf serum. Once cells adhered after the trapping, the media were switched back to those listed previously.

Substrate fabrication, as illustrated in Figure 2, began with the deposition of 50 angstroms of titanium followed by the deposition of 150 angstroms of gold, both at a rate of 1 angstrom/second, onto 22 by 60 mm, #1.5 coverslips. Metal deposition was accomplished via electron beam evaporation. The metal-coated coverslips were treated with plasma in an evacuated plasma etcher for 1 minute, and then treated with 20% hexamethanedisilazane in propylene glycol methyl ether acetate for 1 minute. Next, Shipley 1813 photoresist was spin coated at 2500 rpm for 15 seconds, to achieve a thickness of ~1.2 microns. The resist was baked at 100C for 5 minutes. To generate a pattern, the resist covered with a standard chrome-on-gold mask, having 3 micron circles corresponding to the 3 micron wide electrodes, and exposed to 3 mJ/cm^2 collimated, broadband UV light using a mask aligner. After exposure, the resist was developed in "351" developer, diluted 1:5 in water, for 45 seconds, and then rinsed with water. Substrates were then treated with plasma in an evacuated plasma etcher for 30 seconds.

The gold comprising the traps was then electroplated onto the flat gold layer underneath the photoresist. Electroplating was accomplished using TG25E plating solution from Technics, inc. The plating current was set at 1 mA/cm^2 for 18 minutes. Substrates were then rinsed with water. Cross-linking of the photoresist was achieved by heating the substrates to 160C and simultaneously exposing them to 1.5 mW/cm^2 UV irradiation for 6 minutes. After electroplating, some substrates were coated with a 300 angstrom layer of silicon dioxide, via electron beam evaporation, at a rate of 3.5 angstroms/second.

The adhesiveness of the substrates was patterned, in alignment with the electrodes, by depositing fibronectin or collagen to form adhesive regions and depositing bovine serum albumin or Pluronics F127 (from BASF Corp.) in other areas to form non-adhesive regions. Protein deposition was accomplished via either microcontact printing or adsorption through an elastomeric membrane. Microcontact printing was accomplished via a technique similar to that described by Folch et al [7]. Briefly, a PDMS stamp was affixed to a glass backing to prevent distortion, coated with the protein, and then pressed against the substrate to transfer the protein. Alternately, the substrate was covered with an elastomeric membrane, as described by Duffy et al [8]. Protein was then adsorbed through patterned holes in the membrane. After adsorption, the membrane was removed, leaving protein adsorbed only to the regions not previously covered by the membrane. Membranes were composed of a layer of PDMS, used to reversibly adhere to the substrates, and a layer of epoxy, to prevent distortion.

After adhesive protein was patterned using microcontact printing of adsorption through membranes, areas not covered with protein were rendered non-adhesive by the adsorption of either 0.2% Pluronics F127 or 1% BSA in water for 1 hour.

Electrodes were placed in a parallel plate flow chamber, in which the pattered electrode described above was positioned ~100 microns from an unpattered electrode composed of a layer of gold on glass. This configuration is referred to as "posts and lid" Since the electrodes are in the shape of posts, and the counterelectrode forms the "lid" of the flow chamber. Fluid flow was used both to introduce cells to the substrate, and to remove untrapped cells after DEP trapping was complete. The DEP media, introduced using a 3mL syringe, was gradually increased until untrapped cells were washed away.

Cell health was assessed by comparing the proliferation rates and morphology of cells undergoing DEP with those not undergoing DEP. Phase contrast images of cells were taken using a cooled CCD camera (Spot RT Slider, Diagnostic Instruments) attached to an inverted microscope (Eclipse TE200, Nikon) with a 4X objective. Cells were counted manually from these images.

Electric Fields were modeled using finite element analysis software (FlexPDE 3 by PDE solutions).

Results:

BPAEC's and 3T3 Fibroblast cells were trapped in 2D arrays by DEP with single cell precision (Figure 3). Electrodes were typically energized with 5 volts at 2 MHz, although 1-10 volts at 0.1-10 MHz also provided effective trapping. Other cell types could also be patterned using DEP. Trapping of single cells required electrode dimensions smaller than the cell size (~10 microns). When larger electrodes were used, multiple cells were trapped at each site (Figure 8). However, the exact size of the electrodes was unimportant as long as the width of the electrode was smaller than the diameter of the cell. Other device dimensions such as chamber height, electrode spacing, and electrode height were not critical, although trapping strength increased with decreasing chamber height. Arrays as large as 1cm by 1cm have been constructed, although this size was chosen arbitrarily and does not represent a fundamental or practical limit. Cell trapping on 1cm by 1 cm arrays took approximately 10 minutes.

In some cases, the entire substrate surface was coated with a 300-angstron layer of silicon dioxide, providing a uniform surface chemistry and removing the cells from direct contact with electrodes. To restrict cell location after DEP trapping, the adhesiveness of some substrates was patterned in registration with the DEP traps. The locations and geometries were limited only by the resolution of the technique used to pattern the adhesive protein. Cell positions were confined to adhesive areas, as shown in figure 7.

The health of cells trapped with DEP (5 volts at 2 MHz) was not adversely affected, as seen by other investigators [9]. Proliferation rates were similar among cells trapped with DEP in DEP Media versus untrapped cells cultured in standard culture media (Figure 4). Cell morphologies were also indistinguishable. Since division rates and morphologies are broadly indicative of cell health, we conclude that cells are not adversely effected by DEP trapping or the DEP media.

We have also constructed electrodes with independently switchable sets of traps (Figure 9). Such electrodes are capable of patterning multiple cell populations in order to study heterotypic cell interactions or create functional tissue constructs and cell-based device

Conclusions:

We have demonstrated DEP as a safe and effective means of patterning mammalian tissue culture cells independently from the surface characteristics of the substrate. Cells can be patterned in arbitrary locations with single cell precision. This type of patterning allows greater control over cell placement than previouspatterning schemes, and is particularly powerful for the investing tion of heterotypic cell-cell interactions. Experiments with DEP will also be used to further define the interactions between living cells and artificial surfaces. Ultimately, DEP will not only help to answer fundamental questions in cell biology, but also be central to multiple biotechnology applications. Because DEP is controlled by small electric potentials, it is inherently suited for integration into computer controlled, lab-on-a-chip devices.

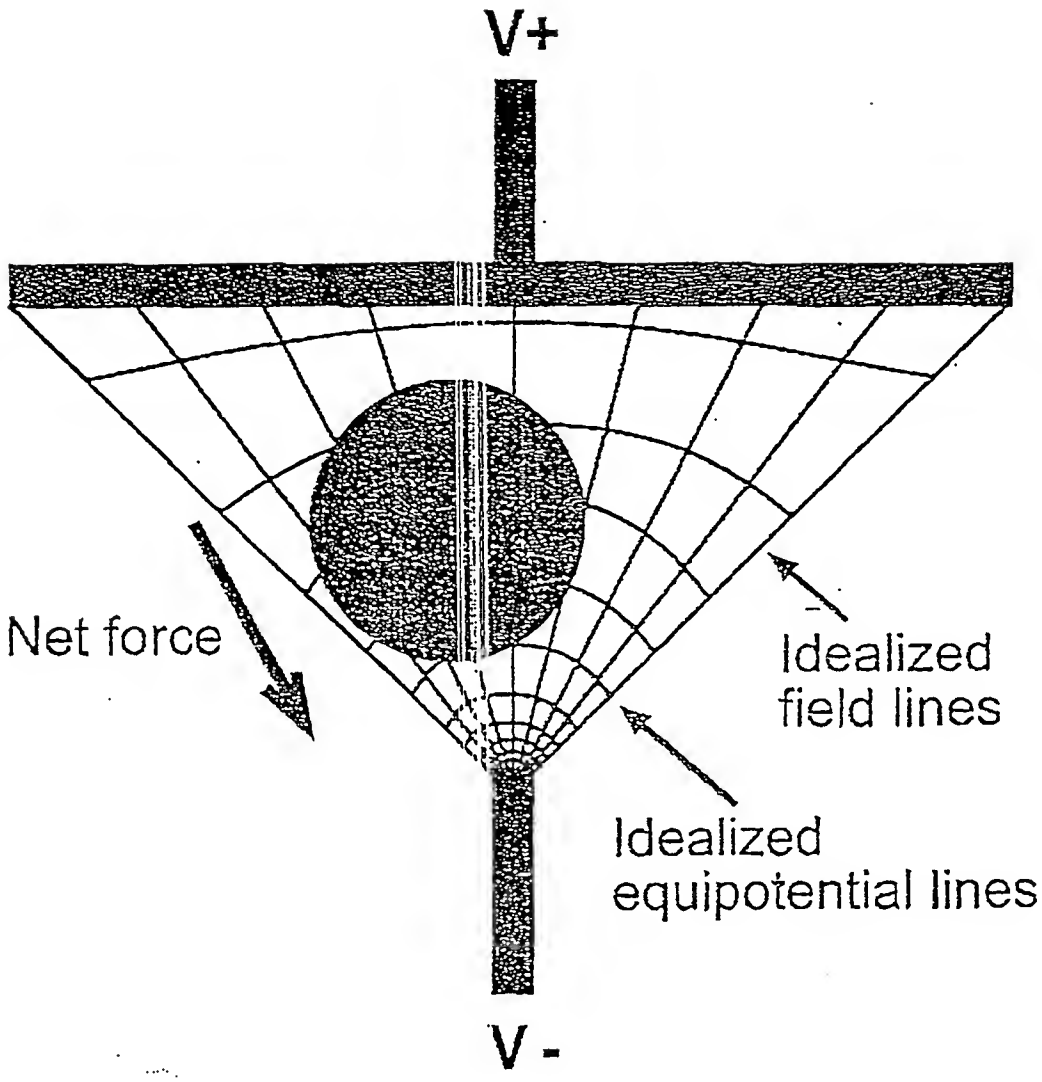


FIGURE 1: The particle, which is more polarizable than the surrounding media, is pulled toward a field maximum. When the signs of the voltages are reversed under AC conditions, the particle feels the same force.

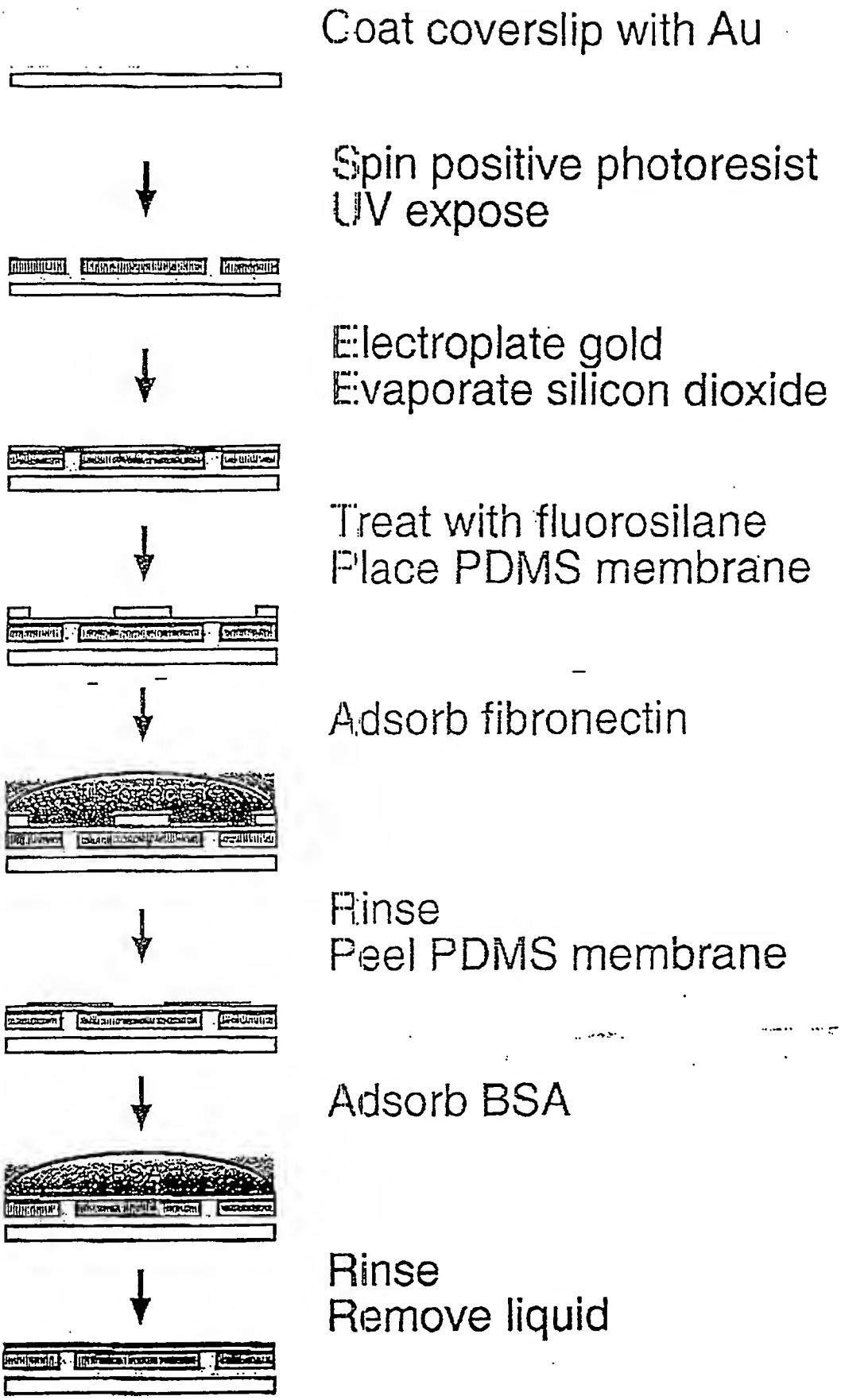


Figure 2: Electrode Array Construction, shown with optional surface patterning using aligned membranes.

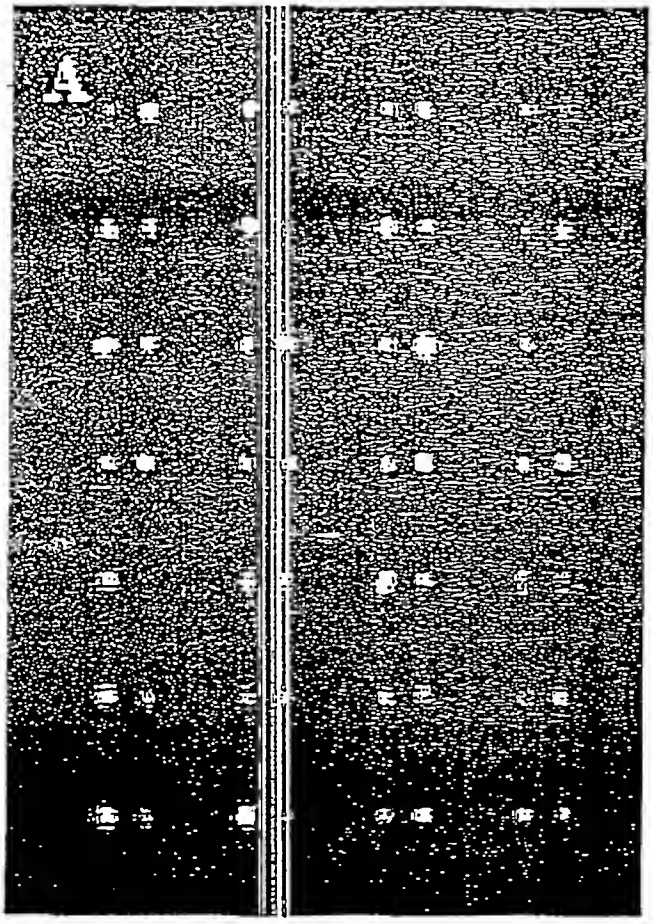


Figure 3: Array of cells trapped using DEP. (top view)

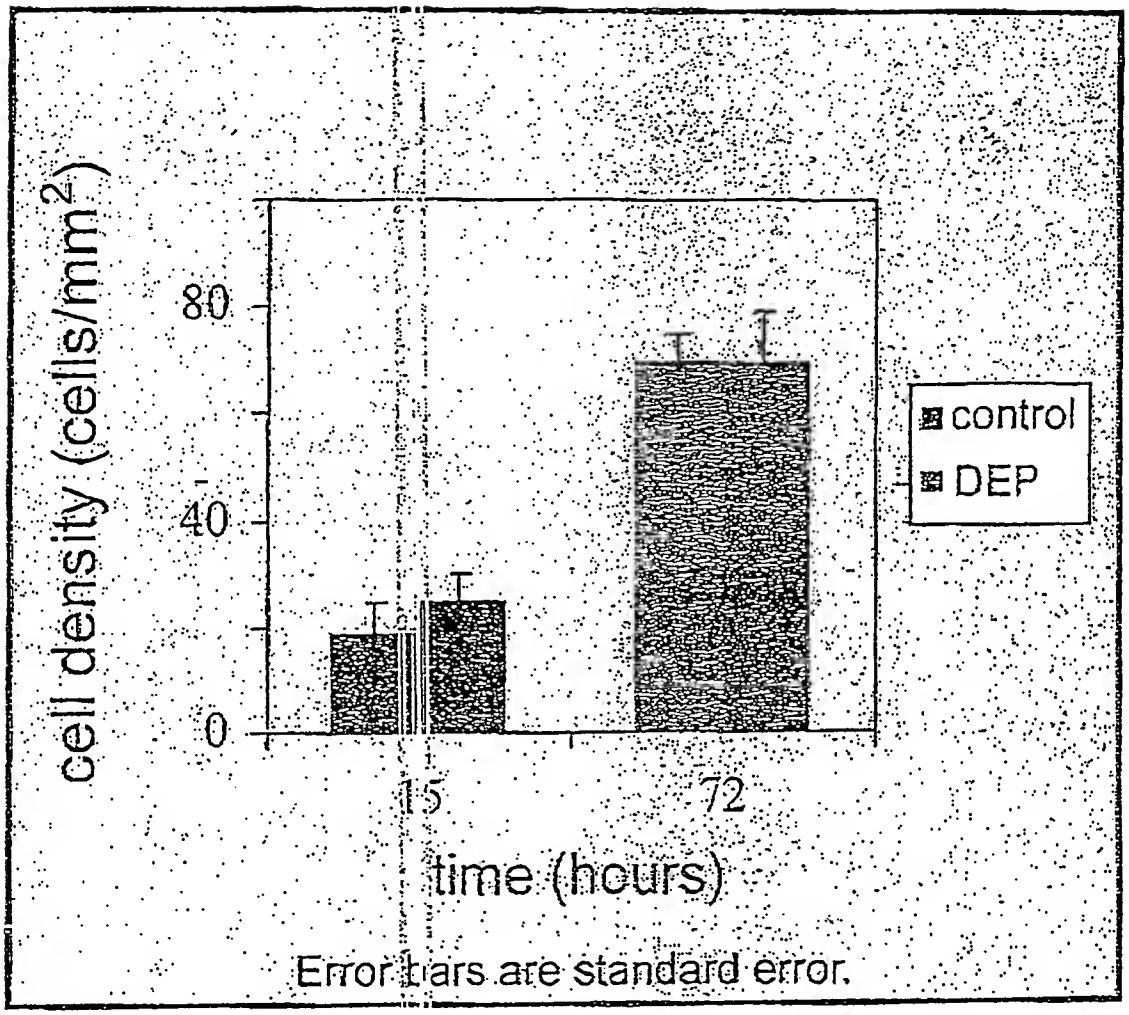


Figure 4: Cell Health: Increases in cell numbers, indicative of cell health, are similar in cells (BPAECs) undergoing DEP in sucrose solution versus control cells in standard tissue culture conditions.

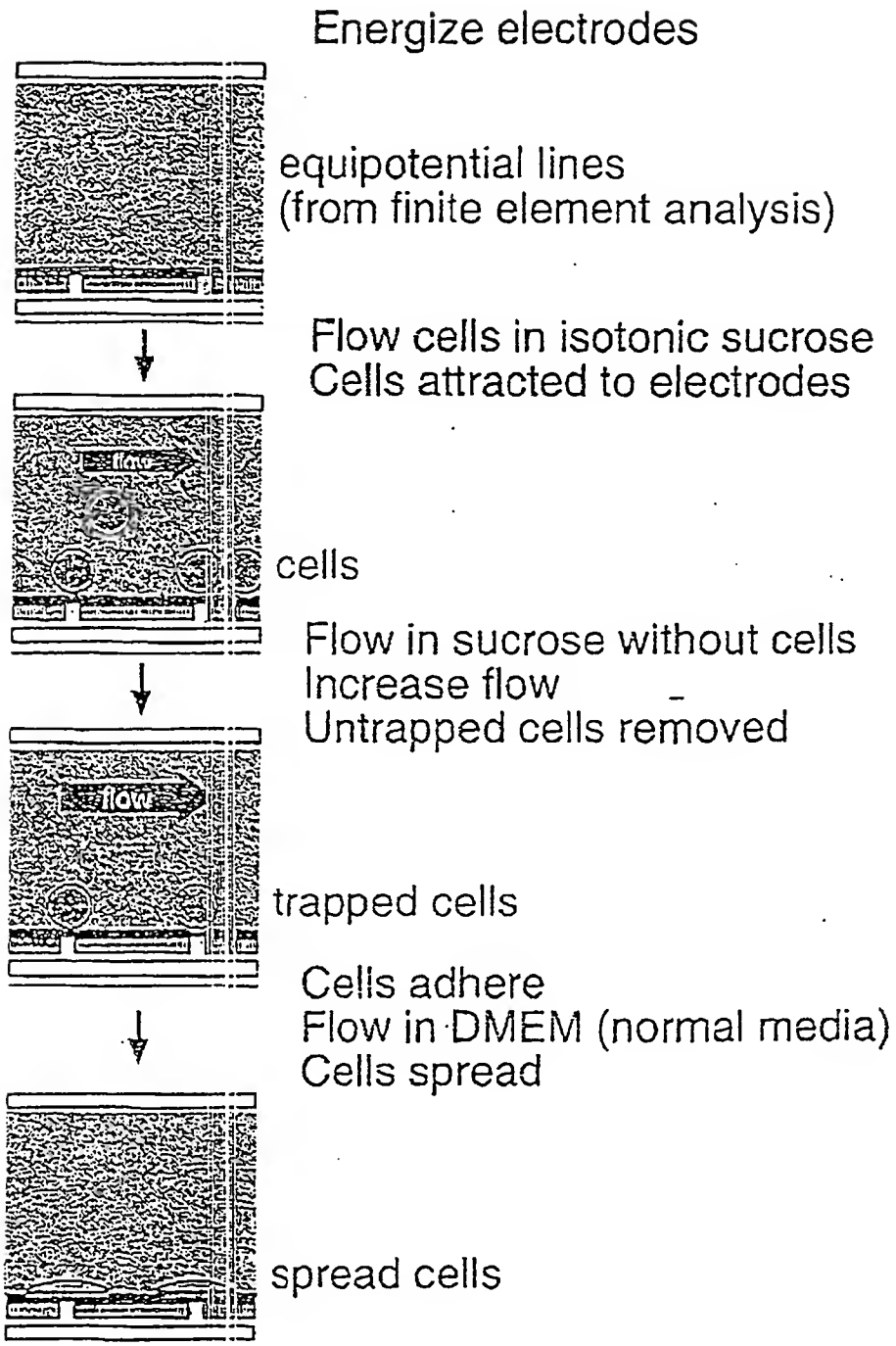


Figure 5: Process of DEP trapping with "post-and-lid" geometry, shown with optional addition of adhesiveness patterning.

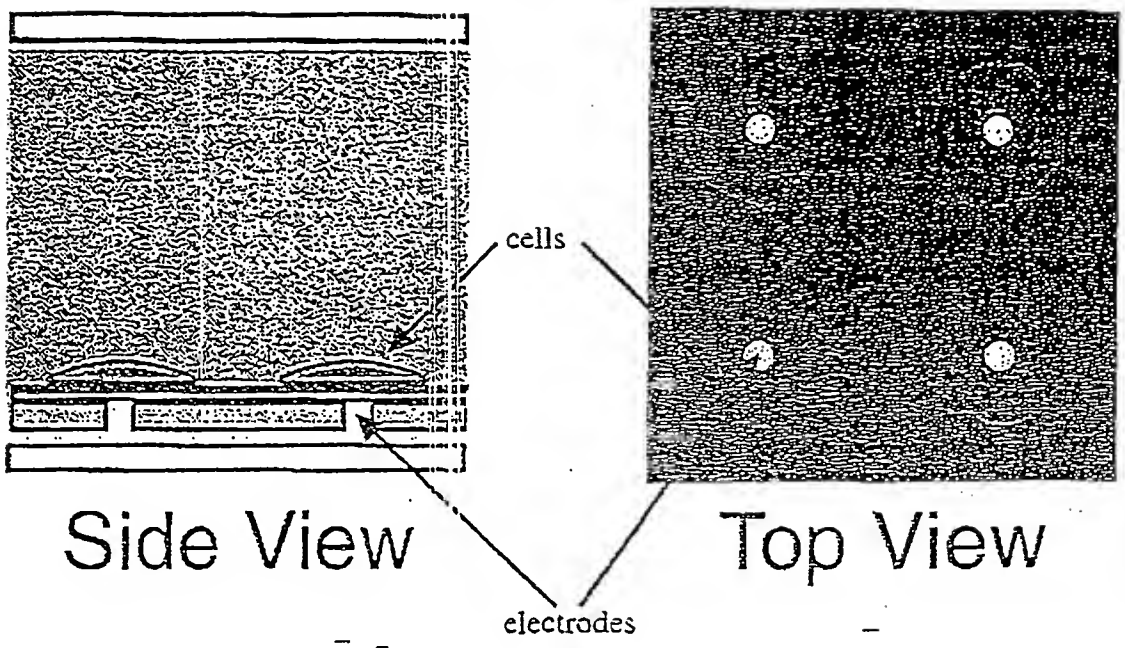
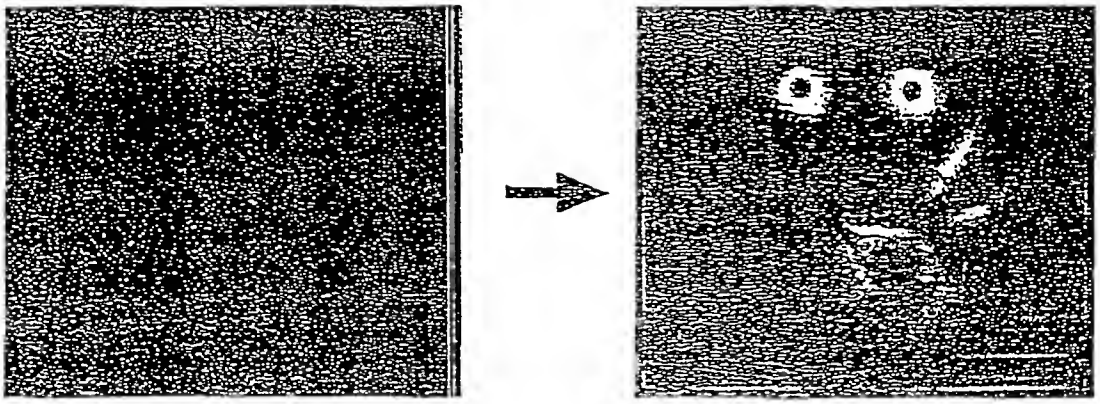
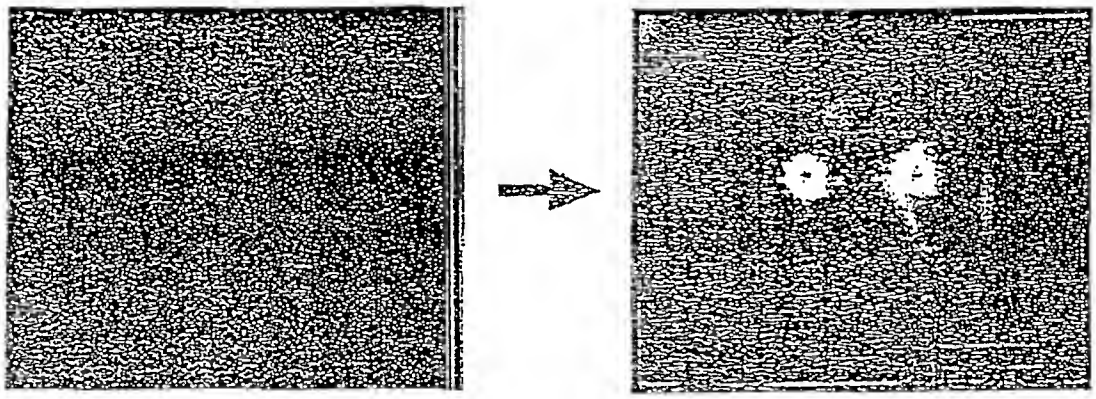


Figure 6: multiple schematic views of cells patterned with DEP



Motile cells patterned with DEP alone migrate away from traps, shown in blue.



Motile cells patterned with DEP and surface chemistry are confined to traps.

Scale bars are 30 μm.

Figure 7: Combining DEP patterning with adhesiveness patterning (top view)

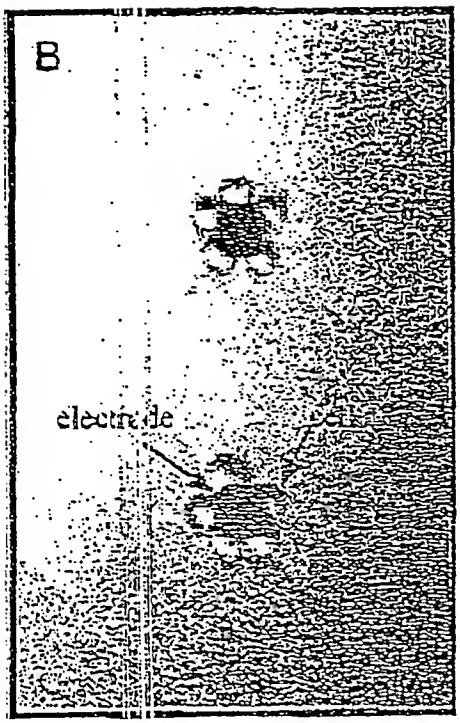


Figure 8: Electrodes larger than 1 cell diameter trap multiple cells.

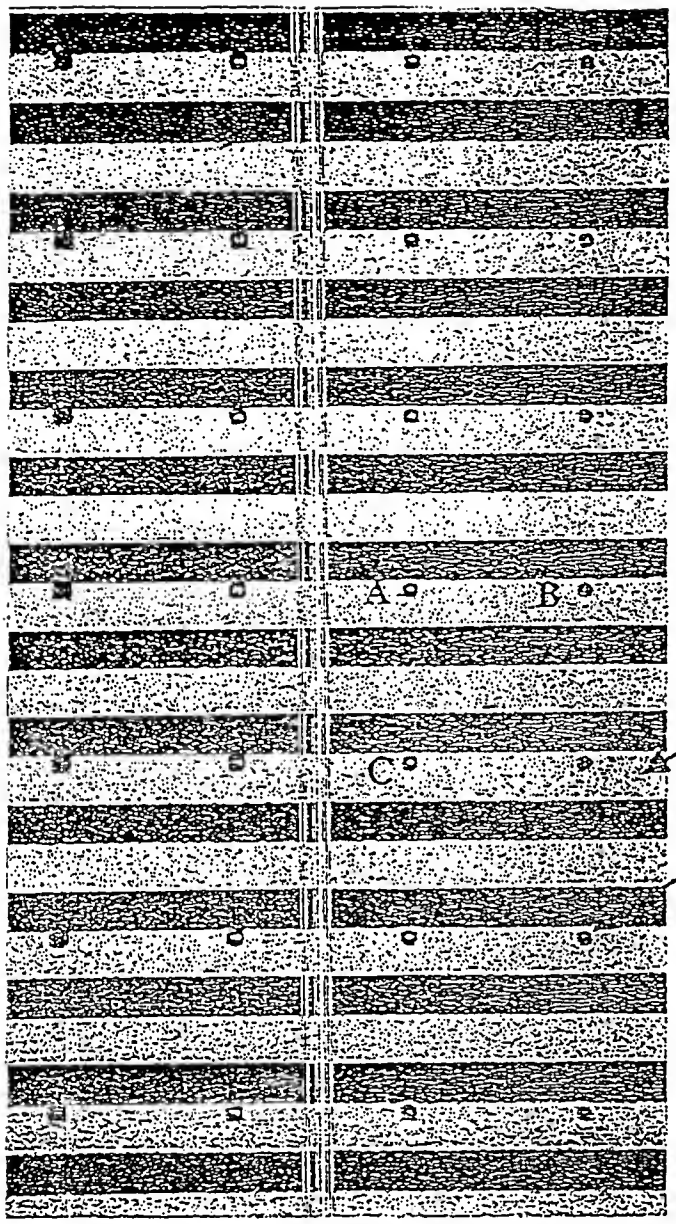


Figure 9: Electrodes (traps) or groups of electrodes with separate electrical connections are independently controllable. The surface of the chip is coated with a clear insulator except at electrode locations. Electrodes A and B are jointly actuated, because they are on the same conductive bus. Electrode C, on a separate bus, is actuated separately from electrodes A and B. Separate connections to individual electrodes are also possible. Buses not containing electrodes are inactive.

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All publications, patents and patent applications disclosed herein are incorporated into this application by reference in their entirety.

For example: "Sambrook et al, Molecular Cloning, A Laboratory Manual (volumes I-III) 1989, Cold Spring Harbor Laboratory Press, USA" and "Harlowe and Lane, Antibodies a Laboratory Manual 1988 and 1998, Cold Spring Harbor Laboratory Press, USA" provide sections describing methodology for antibody generation and purification, diagnostic platforms, cloning procedures, etc. that may be used in the practice of the instant invention.

The following claim(s) of this provisional application are not to be construed as limiting the disclosed invention(s). The claim(s) are included for compliance with patent application structural regulations that may be imposed by international patent offices.

We claim:

1. A method of forming an array of 3T3 cells in a 2D array onto the construction of Figure 2 using the procedure of Figure 1.